Monitoring the Adult Navel Orangeworm Moths with Pheromone and Host-Plant Volatiles

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Objectives:

1. Compare in a wind-tunnel assay the in-flight and close-range male orientation and courtship behaviors evoked by point-source formulations of pheromone, including dispensers prepared for evaluation in the field. Determine which blend of components evokes the highest proportion of source finding.
2. Use the wind-tunnel assay to determine why some batches of synthetic pheromone and formulated lures are more attractive than others. In collaboration with Brad Higbee, field test formulated dispensers of pheromone for efficacy.
3. Determine the pattern of pheromone dispersal, with particular attention to vertical movement of odor plumes, using the almond orchard as a model habitat. These measurements will aid in determining the optimal height in the canopy for placement of monitoring traps and puffers.
4. Develop behavioral bioassays to evaluate the attractiveness of host plant volatiles to mated females and as synergists for the pheromone. Test identified host volatiles supplied by John Beck for attractivity.

Interpretive Summary:

We have two overarching goals: 1) to improve the efficacy of mating disruption in the navel orangeworm moth, *Amyelois transitella*, referred to as "NOW." 2) To aid in the development of a highly attractive lures that would be useful as a monitoring tool in pest management programs. To these ends we have: 1) Optimized the composition and ratio of components for maximal male attraction in a wind tunnel. 2) Shown that four expected breakdown products of the main aldehyde pheromone component do not affect attraction. Similarly shown that the two isomers of the alcohol components also do not diminish attraction. 3) Tested a number of lures formulated to provide longevity in the field and competitiveness with female-baits. 4) Established the patterns of pheromone dispersal in almond orchards using visual tracers and measured wind flow and turbulence patterns with a sonic 3-D anemometer. 5) Developed new laboratory assays that permits us to show female attraction to candidate synthetics identified from almonds and to test if these chemicals synergize male attraction to pheromone.

One of our goals has been to verify which of the 9 published compounds (as well as others newly discovered but unpublished) mediate attraction and courtship. We have now defined an optimal blend of 4 components (Kanno et al. 2010; Kuenen et al. 2010). Such information is a crucial step in devising highly attractive lures for monitoring traps. Furthermore, the complete pheromone blend should be the most efficacious mixture for mating disruption (Minks and Cardé 1988, Cardé and Minks 1995; Cardé 2007).

Results and Discussion:

1. Upwind flight along the pheromone plume and landing on the odor source required the simultaneous presence of two components, (11*Z*,13*Z*)-hexadecadienal, (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-tricosapentaene, and the addition of either (11*Z*,13*Z*)-hexadecadien-1-ol or (11*Z*,13*E*)-hexadecadien-1-ol. A mixture of all 4 components produced the highest levels of rapid source contact. In wind-tunnel assays, males did not distinguish among a wide range of ratios of any of the three components added to (11*Z*,13*Z*)-hexadecadienal. Dosages of 10 and 100 ng of the 4-component blend produced higher levels of source location than dosages of 1 and 1000 ng (Kanno et al. 2010; Kuenen et al. 2010). The broad range of component ratios that evoke attraction has simplified some aspects of development of a field lure.

2. So far six possible contaminants have been evaluated for their possible suppressive effect on attraction. These compounds were prepared and supplied courtesy of Bedoukian Research. Adding any of the isomers (the 11Z, 13E, the 11E, 13Z, and the 11E, 13E) of the main pheromone component to the complete blend did not diminish attractiveness. Work with many other moth species often has found that geometrical isomers of monounsaturated or doubly unsaturated pheromones are often inhibitory to attraction, unlike NOW. Similarly, adding the acid of the aldehyde to the 4-component blend also did not affect attractiveness. Isomers of the two alcohols (of the two alcohol pheromone components) also did not diminish attraction. This is very helpful to lure formulation, in that all of these breakdown products/ or synthetic contaminants could be expected in lures.

A second approach has been to compare lures using the principal pheromone component (the aldehyde) from different sources, or following purification, while holding the other 3 components constant. Clearly batches have differed somewhat differ in their attractively, but the chemical identity of the antagonist(s) remains unclear.

Work on lures for the field has relied on membrane dispensers prepared by Suterra. These have been evaluated in the field by Brad Higbee and in our wind tunnel. In brief, the new Suterra membrane formulation is effective in the field for 4 weeks, although it is not quite as attractive as females. As well, the 4-component formulation outperformed the 3-component blend (Higbee et al. 2014).

3. Nighttime observations in almond orchards found that pheromone plumes have considerable vertical movement. We documented this dispersal pattern using a high resolution, 3-D sonic anemometer at 6 heights from 2 to 7 meters (2 meters above the canopy). We found a net upflow of air during the time when NOW mate (late night to dawn) and substantial turbulent mixing within the canopy (Girling et al. 2013).

One practical implication of these observations for mating disruption is that when pheromone puffers are deployed at height of the top of the orchard canopy, as is current practice, much of their output may be carried upward out of the canopy. This shows that much of the output of puffers so deployed would be unavailable to disrupt NOW mate finding—that is, it would be "wasted." We suggest that puffers would be just as efficacious if deployed and mid canopy. As a result of these observations and his own work, Brad Higbee has evaluated male capture in female-baited traps positioned at several canopy heights and the effect of the height of puffers on the efficacy of mating disruption.

4. We use a large (1.8 m high by 1.5 wide) cage housed in an environmental room to assay the attraction of mated NOW females to natural oviposition substrates (e.g., almond meal) and also synthetic host compounds supplied by John Beck. This method shows that females orient to host odors (compared to unbaited controls). Blends or individual compounds supplied by John Beck (see for example Beck et al. 2012) are being tested for attractivity. The general setup is depicted in Fig. 1. This system has the advantage of allowing assay of candidate compounds or blends year-round.

5. We also are evaluating host volatiles in our wind tunnel for their potential as synergists for male attraction to pheromone. Volatiles are added to the pheromone plume and attraction levels are compared to pheromone alone and volatiles alone. Adding host odors to pheromone has improved codling moth monitoring and also shows promise for many other moth pests (reviewed by Deisig et al. 2014).

Figure 1. Bioassay system used to test attractivity of natural and synthetic odor sources for female attraction for oviposition. Compounds supplied by John Beck. The cage rotates to avoid position effects. The insets show the capture devices that moths must enter to be considered as "attracted."

Research Effort Recent Publications:

Girling, R.D. and R.T Cardé. 2006. Analysis of the courtship behavior of the navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), with a commentary on methods for the analysis of sequences of behavioral transitions. J. Insect Behav. 19:497-520.

Kanno, H., L.P.S. Kuenen, K.A. Klingler, J.G. Millar and R.T. Cardé. 2010. Attractiveness of a four-component pheromone blend to male navel orangeworm moths. J. Chem. Ecol. 36:584-591.

Wang, H.-L., C.-H. Zhao, J. G. Millar, R.T. Cardé and C. Löfstedt. 2010. Biosynthesis of unusual moth pheromone components involves two distinctly different pathways in the navel orangeworm, *Amyelois transitella*. J. Chem. Ecol. 36:535-547.

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Minks, A.K. and R.T. Cardé. 1988. Disruption of pheromone communication in moths: is the natural blend really most efficacious? Entomol. Exp. Appl. 49:25-36.

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